Dear Prof. Michael Weintraub,

Thank you very much for your helpful comment to our manuscript, and we have carefully and thoroughly revised the manuscript according to your and referees' comments. The detailed responses to the comments are as follows.

Response to Anonymous Referee #1

The authors have made substantial progress. Here I note the some comments that are, in my view, still not addressed adequately.

Response: We thank referee for the helpful comments. After discussing with co-authors, we thoroughly revised the manuscript and listed in supplement.

Correction factors: it's the 0.45 and 0.54 (now page 8, L8). I recommend not correcting these numbers using these factors.

Response: Thank you for your comment. But, we thought correction factors of 0.45 for C and 0.54 for N were reasonable for the determination of MBC and MBN.

There is still no reason given for the pseudoreplicative design (ie three non-independent sets of 5 samples that are then treated as independent). It's not ideal, but there are often acceptable reasons for it and so it's not a fatal flaw in the study. However, I'd like the know the authors' thinking here. The way I see it there are two possibilities: (1) logistically it was not possible to randomly sample across the entire area (meadow or meadow system) of interest, but the authors wanted 15 samples so this is how they were able to achieve that. (2) they were originally planning on comparing the sites, but there were not a lot of interesting differences so they pooled them together in order to achieve

a better overall understanding of seasonal trends, which were perhaps not visible with n = 5 at each site. This was a point brought up by multiple reviewers and the authors need to provide a justification on why such a design was used.

Response: Thank you for your comments. Actually, we have explained why we selected 3 adjacent sites that because of the consideration of the soil spatial heterogeneity in the alpine meadow. Furthermore, the 15 samples at each sampling time were independent, because they were randomly collected at different locations **(Page 6 lines 2-5)**.

Figures: having the bar graphs below the line graphs further emphasizes that the information is redundant. I'll leave it up to authors and editor, but I still recommend removing the bar graphs.

Response: Thank you for your comment. But, we did not think having the bar graphs below the line graphs further emphasizes that the information is redundant. Because, they showed different information, i.e., the line graphs showed detailed information on intra- and interannual patterns of microbial and nutrient dynamics; the bar graphs showed significant differences in microbial biomass and nutrients between seasons and years, and their interaction effects. Thus, these figures were indispensable for our study.

Inclusion of data in a publicly available repository: the authors did not address this request. I still recommend it.

Response: Yes, The data set related to this study has been provided as a supplement.

Re: TDN vs. MBN numbers. Some ok points made in the reviewer response, but it's not in the paper. It warrants discussion in the paper.

Response: Thank you for your comment. Actually, the important points made in the response have been stated in the discussion section **(Page 17 lines 1-5; Page 18 lines 8-13)**.

Photograph and map are not the same thing. It helps to see what the

ecosystem looks like on the ground. I still recommend this, even if in the supp materials.

Response: Sorry, we did not have appropriate photographs to show the alpine meadow.

A comment from another reviewer about unsubstantiated statements in the discussion is still relevant: "Finally, there isn't direct support for many of the overall conclusions of the paper – this study can describe correlations, but not the types of conclusions described (e.g. soil microorganisms play a crucial role in accumulation of inorganic N pools)" Some examples of these statements:

"This period of active microbial activity and N mineralization benefited from substrates conducive for microbial growth, particularly those supplied by the fresh plant litter inputs in autumn."

Response: Thank you for your comment. Here, we did not make any conclusion, but only cited some relevant results of previous researches. We revised "benefited from" as "might benefit from" **(Page 14 line 8)**.

"Snow melting during this period is an important source of NH4 +–N" This is only true with high deposition. Not sure if this region is susceptible to that.

Response: Thank you for your comment. Actually, this sentence was also a citation of previous research, which is a potential reason why "A trend of increasing NH_4^+–N content was found during the early soil thaw". We revised "is an" as "may be an" **(Page 16 line 14)**.

"At the start of the growing season, NH4 +–N content sharply decreased, partly because alpine meadow plants prefer NH4 +–N" maybe change partly to possibly

Response: Yes, "partly" has been changed into "possibly" **(Page 16 line 6, 15; Page 18 line 13)**.

"the seasonal dynamics of different available N pools showed

significant complementarity with the nutrient supply process and play a crucial role in maintaining abundant biodiversity of alpine meadow ecosystem"

Response: Yes, we have revised “and play” as “and will play”, and some relevant references were added **(Page 17 line 6)**.

"However, they showed a divergent interannual pattern during the growing season, partly because of the plant and microbe uptakes and leaching effects." same use of partly. maybe authors mean to use possibly in these cases.

Response: Yes, “partly” was changed into “possibly”.

"According to our monitoring results, soil temperature and water condition are the primary environmental factors driving the seasonal and interannual dynamics of soil microbial biomass and available N pools." I would probably leave this out. It's something most would accept but at the same time, is not really shown by this study, which does not address mechanisms.

Response: Thank you for your comment. But, we thought this conclusion could be made according to our results **(e. g., Figures 2, 3; Tables 1, 2)** that “soil temperature and water condition are the primary environmental factors driving the seasonal and interannual dynamics of soil microbial biomass and available N pools”.

Finally, I would still axe the mentions of climate change from the conclusion. The contributions of this paper are on seasonal trends, not climate change. It's not a huge change. It's fine to mention it where it is mentioned just before the conclusion, but it should not be emphasized as the final statement in the paper. The authors justification in the response that because temperature and moisture appear to correlate with the other measured variables, climate change is important is not convincing. Temperature and moisture are always

important for microbial processes, and it's a leap to then suggest that this paper provides particular insight on how this relationship will change with climate.

Response: Thank you for your comment. But, we still thought it was necessary to mention the climate change in the conclusion. Because temperature change and uneven distribution of precipitation are two important aspects of climate change. Furthermore, the alpine ecosystems are sensitive to the future climate change.

minor:

"An obviously trend of increasing NH4 + –N content was found during the early soil thaw" this revised sentence does not make sense

Response: Thank you for your comment. But we thought this sentence was important, because it showed a pulse phenomenon of NH_4^+–N during the late nongrowing season, which might play a crucial role in nutrient supplies for plants during the early growing season.

Response to Anonymous Referee #2

I think that thanks to the suggestions of the referees the paper has considerably improved. However I suggest to the authors some further changes, listed below:

Pag. 27 line 7: change avilable into available. See also pag 27 line 10.

Response: Yes, "avilable" was changed into "available" **(Page 4 lines 3, 6)**.

Pag 28 lines 6-7: Did you add also the soil classification according to the Soil Taxonomy (Silty Loam Inceptisol)? If yes please add the proper reference: Soil Survey Staff. 2014. Keys to Soil Taxonomy, 12th ed. USDA-Natural Resources Conservation Service, Washington, DC.

Response: Yes, the reference that "Soil Survey Staff: Keys to Soil Taxonomy, 12th ed. USDA-Natural Resources Conservation Service, Washington, DC., 2014." was added **(Page 5 line 18; Page 26 lines 6-7)**.

Pag 28 lines 10-11: Please specify the elevation of the 3 sites

Response: Sorry, we only measured the elevation of the center site, because the 3 sites were adjacent, and their differences in elevation were very small.

Pag 29 line 1: add O horizon before living plant roots and litter

Response: Yes, "O horizon" was added before "living plant roots and litter" **(Page 6 line 11)**.

Pag 35 line 14: sorry I don't understand this sentence. What do you mean with "from each other"

Response: "from each other" means that significant differences compared with each other. We have deleted "from each other" in the revised manuscript **(Page 12 line 12)**.

Pag 35 line 18: What do you mean woth the term those

Response: Sorry, we could not find "those" in Page 35 line 18, we were not sure which "those" in the manuscript did you refer.

Pag 37 line 10: delete early

Response: Sorry, we could not find "early" in Page 37 line 10, we were not sure which "early" in the manuscript did you refer.

Pag 38 line 18: what do you mean with a-1

Response: The "a^{-1}" in the units for biomass refers to per year.

Pag 40 line 1: Why obviously?

Response: "obviously" was deleted **(Page 13 line 10; Page 16 line 11)**.

Pag 43 line 5: Delete monitoring

Response: Yes, "monitoring" was deleted **(Page 18 line 5; Page 19 line 11)**.

Response to Anonymous Referee #4

This paper describes both seasonal and interannual variability in soil microbial biomass and soil available N in alpine tundra with monthly

resolution over a 3 year time period. This is an impressive data set which is worthy of publication. I reviewed an earlier version of this manuscript and made a number of suggestions. The writing is much improved but still requires further work. Further, some of my previous comments have not been dealt with to my satisfaction, as described below.

Response: We thank referee for the helpful comments. After discussing with co-authors, we thoroughly revised the manuscript and listed in supplement.

In the previous version I had questions about the MBC/MBN methods as well as the statistics. Neither of these have been dealt with satisfactorily. The methods for determining TDN are still not clear. The procedure for TDN is described on page 7 line 11-16. Line 9-11 describes the chloroform fumigation procedure which is not the methods for TDN but are part of the methods for MBN. TDN is determined on both fumigated and non-fumigated samples (the non-fumigated sample analysis is currently not described in the paper) and the difference is MBN. The chloroform fumigation methods could be moved to the paragraph beginning on page 8 line 6.

Response: Yes, the methods for determining TDN, MBC and MBN were rewrote according to your suggestion (**Page 7 lines 8-16; Page 8 lines 7-17**).

For the statistics, the response to reviewer 4 indicates that sample ID was included in the model to account for the lack of independence of samples across time. However, the stastistical analysis section of the manuscript does not describe any inclusion of sample ID in the model. Please clarify in the manuscript. Also, all F values throughout the manuscript should include the degrees of freedom.

Response: Thank you for your comment. Actually, we have described the

sample ID before the description of the mixed-effects model, i.e., "Microbial and nutrient variables were analyzed to test the intra-annual differences between the growing season (i.e., data from May to October were used as a sample set; n = 90) and nongrowing season (i.e., data from November to April were used as a sample set; n = 90)" **(Page 9 lines 15-18)**.

Yes, the degrees of freedom (df) were added behind the *F* values throughout the revised manuscript **(Page 11 lines 5, 15-16; Page 12 lines 11-12; Page 13 lines 1-2, 8-9, 14-15)**.

Some parts of the result section are also not clear. For example, when describing DOC patterns, the seasons are described as being not significantly different from each other (page 11 line 18) and also significantly different (page 12 line 4).

Response: Thank you for your comment. Although, "the seasonal dynamics of DOC had no significant difference", the interaction effects of DOC between season and year were significantly different. Thus, "the DOC contents during the nongrowing season in 2011–2012 (174.27 mg kg^{-1} ± 32.59 mg kg^{-1}) and growing season in 2012–2013 (170.85 mg kg^{-1} ±41.19 mg kg^{-1})" showed the result that "were significantly lower than that in other seasons".

Also, the results section could be reduced – e.g. The two sentences from page 10 line 17 to page 11 line 1 say the same thing.

Response: Thank you for your comment. Actually, the two sentences described different things, i.e., the first sentence showed that "the MBC values in the nongrowing seasons were consistently higher than those in the growing seasons"; the second sentence showed that "the mean MBC value during the nongrowing season in 2012–2013" was the highest among different seasons.

Finally, just as a suggestion, figure 3 could also be presented with only

part A and shading to indicate the different season – this way the data is only presented once rather than repeated in both parts of the figure. The same change could be applied to the other figures. The two types of presentations are presented in the same figure sometimes (Figure 3) and as separate figures in others (Figure 7 and 8).

Response: Thank you for your comment. But, we thought these figures were indispensable for our study. Because, they showed different information, i.e., the line graphs showed detailed information on intra- and interannual patterns of microbial and nutrient dynamics; the bar graphs showed significant differences in microbial biomass and nutrients between seasons and years, and their interaction effects.

In the discussion, the authors still need to be cautious about implying causality for some of the patterns they have measured. Some examples are below:

Page 13 Line 14 – indicate that the mechanism for the increase in soil MBC and available N are speculative. Also, the conclusion of this paragraph describes an accumulation of organic N which is not described in the remainder of the paragraph.

Response: Yes, this sentence was deleted **(Page 14 lines 12-14)**.

Page 15 Line 15 – Only describe what you have evidence for – e.g. an increase in microbial biomass and not activity

Response: Yes, the microbial activity was revised as "microbial biomass" **(Page 16 line 9)**.

And lastly, a few clarifications are required in the discussion:

Page 15 line 9 – what do you mean by the "number of bacteria" increased just after thaw? The previous paragraph describes a crash in microbial biomass. Do you mean the proportion of bacteria to fungi? The number of bacterial phenotypes?

Response: Here, the "number of bacteria" mean the number of CFU of

bacteria.

Page 15 line 2-5 The units for soil organic matter need an area (per m2?) for this comparison to be relevant. Is this sentence implying that the SOM in tundra is limiting to microbial growth in some circumstances?

Response: Thank you for your comment. But, we thought the units for soil organic matter did not need an area, because we just need compare the differences of soil organic matter contents among different alpine meadows. Furthermore, we also did not know the values of SOM per square meter (m^2) from the references. According to this sentence, we knew that the SOM contents in the alpine meadows of the Qinghai-Tibet Plateau were relatively higher than that in other alpine meadows. It was implied that the available C was relatively sufficient in this region and "might not restrict microbial activity during the winter–spring transition" **(Page 11 lines 9-10)**.

The writing in this version is much improved over the last version. However, the writing still needs to be improved for clarity and grammar. A number of examples follow:

Page 1 Line 10 – replace "occurs seasonally" with "varies seasonally" as the activity occurs all the time

Response: Yes, "occurs seasonally" was replaced with "varies seasonally" **(Page 1 line 10)**.

Page 1 Line 15 – replace "Topsoil samples were" with "Soil was" and "and were analyzed" with "and analyzed"

Response: Yes, "Topsoil samples were" and "and were analyzed" were replaced with "Soil was" and "and analyzed", respectively **(Page 1 line 15)**.

Page 1 Line 17 – replace "was measured" with "as measured"

Response: Yes, “was measured” was replaced with “as measured” **(Page 1 line 17)**.

Page 1 Line 18 – replace “the number of” with “the proportion of”

Response: Yes, “the number of” was replaced with “the proportion of” **(Page 1 line 18)**.

Page 2 Line 1 – replace “induced by soil temperatures” with “induced by changes in soil temperatures”

Response: Yes, “induced by soil temperatures” was replaced with “induced by changes in soil temperatures” **(Page 2 line 2).**

Page 3 Line 11 – delete “apparently”

Response: Yes, “apparently” was deleted **(Page 3 line 11)**.

Page 4 Line 5 Delete “In alpine systems” as it is repeated later in the sentence

Response: Yes, “In alpine systems” was deleted **(Page 4 line 5)**.

Page 5 line 8 Delete “and”

Response: Yes, “and” was deleted **(Page 5 line 8)**.

Page 6 line 10 – Delete “and 15 soil samples… time” as this information is repeated from a few sentences earlier

Response: Yes, “and 15 soil samples… time” was deleted **(Page 6 line 10)**.

Page 9 line 4 – “analysis of variance” not “variance analysis”

Response: Yes, “variance analysis” was revised as “analysis of variance” **(Page 9 line 15)**.

Page 14 line 17 – what does the a refer to in the units for biomass? Per year?

Response: Yes, the “a^{-1}” in the units for biomass means “per year” **Similarly, the use of appropriate references is also much improved. A few more which need to be changed are:**

Page 3, line 3: Mikan is not an alpine reference

Response: Yes, “Mikan et al., 2002” was deleted and two relevant alpine references were added, i.e., “Brooks et al., 1996; Jefferies et al., 2010” **(Page 3 line 3)**.

Page 16 line 13 – Qin and Petechey/Gaston are general biodiversity – ecosystem functioning researchers and not appropriate for use in this sentence.

Response: Yes, “Qin et al., 2003; Petchey and Gaston, 2006” were deleted, and two appropriate references were added, i.e., “Kahmen et al., 2006; Ashton et al., 2010” **(Page 17 lines 7-8)**.

Best regards!

Bo Xu

Seasonal and interannual dynamics of soil microbial biomass and available nitrogen in an alpine meadow in the eastern part of Qinghai–Tibet Plateau, China

Bo Xu[2], Jinniu Wang[1, 3], Ning Wu[1, 3], Yan Wu[1, 3], Fusun Shi[1, 3]

[1] Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China.

[2] College of Resources and Environment, Aba Teachers University, Aba, Sichuan 623002, China.

[3] Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization & Ecological Restoration Biodiversity Conservation Key Laboratory of Sichuan Province, Chinese Academy of Sciences, Chengdu 610041, China.

Correspondence to: Fusun Shi (shifs@cib.ac.cn)

Abstract. Soil microbial activity varies~~occurs~~ seasonally in frozen alpine soils during cold seasons and plays a crucial role in available N pool accumulation in soil. The intra- and interannual patterns of microbial and nutrient dynamics reflect the influences of changing weather factors, and thus provide important insights into the biogeochemical cycles and ecological functions of ecosystems. We documented the seasonal and interannual dynamics of soil microbial and available N in an alpine meadow in the eastern part of Qinghai–Tibet Plateau, China between April 2011 and October 2013. Soil was ~~Topsoil samples were~~ collected in the middle of each month and ~~were~~ analyzed for water content, microbial biomass C (MBC) and N (MBN), dissolved organic C and N, and inorganic N; soil microbial community composition ~~was~~ as measured by the dilution-plate method. Fungi and actinomycetes dominated the microbial community during the nongrowing seasons, and the proportion~~number~~ of bacteria increased considerably during the early growing seasons. Trends of consistently increasing MBC and available N pools were observed during the

nongrowing seasons. MBC sharply declined during soil thaw and was accompanied by a peak in available N pool. Induced by changes in soil temperatures, significant shifts in the structure and functions of microbial communities were observed during the winter–spring transition and largely contributed to microbial reduction. Divergent seasonal dynamics of different N forms showed a complementary nutrient supply pattern during the growing season. Similarities between the interannual dynamics of microbial biomass and that of available N pools were observed, and soil temperature and water condition were the primary environmental factors driving interannual fluctuations. Owing to the changes in climate, seasonal soil microbial activities and nutrient supply patterns are expected to change further, and these changes may have crucial implications for the productivity and biodiversity of alpine ecosystems.

1 Copyright statement

We agree with the copyright policy of *Biogeosciences*.

2 Introduction

In Arctic and alpine ecosystems, soil microbial activity plays a crucial role in soil C and N cycles and nutrient transformation in frozen soils during cold seasons (Lipson et al., 1999; Murata et al., 1999; Panikov et al., 2006; Larsen et al., 2007; Matthew Robson et al., 2010). Unfortunately, information on belowground microbial activities and nutrient cycles in both growing and nongrowing seasons in such ecosystems are limited.Moreover, intra-annual biogeochemical cycles affected by the changes in seasonal weather factors in frozen regions are not fully understood. The integration between the intra- and interannual patterns in soil microbial and biogeochemical dynamics has important implications to the exploration of the current and future impacts of climate change on the functions of cold ecosystems (Edwards and

Jefferies, 2013).

Microorganisms in alpine environments covered seasonally with snow can survive in thin unfrozen water films when most of the soil water is frozen (Brooks et al., 1996; Jefferies et al., 2010~~Mikan et al., 2002~~). Previous studies indicated that substantial microbial activity exists in the frozen soils during cold seasons even at temperatures lower than −5 ℃ (Brooks et al., 1996; ~~Lipson et al., 2002;~~ Edwards et al., 2006; Panikov et al., 2006; Jefferies et al., 2010). Although microbial activity is limited by cold temperatures and substrate transport (Deming, 2002; Lipson et al., 2002; Oquist et al., 2009), its cumulative effects on organic matter decomposition in soil during long cold seasons significantly influence annual N pools in Arctic and alpine ecosystems (Lipson et al., 1999; Schmidt and Lipson, 2004; Schmidt et al., 2007; Buckeridge and Grogan, 2008). Thus, by understanding microbial activities in winter, we can broaden our current knowledge regarding nutrient supplies for plants and microbes during the subsequent growing season.

Previous studies suggested that the fungal/bacterial ratio of a soil microbial community in winter is ~~apparently~~ higher than that in summer (Lipson et al., 2002; Schadt et al., 2003), and significant shifts in microbial community structures and functions occur during soil thawing in Arctic and alpine tundras (Lipson et al., 2002; Schadt et al., 2003; Lipson and Schmidt, 2004; Buckeridge et al., 2013). Apart from these changes, the rate of microbial biomass turnover increases during winter-spring transition periods (Edwards et al., 2006; Schmidt et al., 2007; Edwards and Jefferies, 2013; Buckeridge et al., 2013). Furthermore, available C substrates for microbial communities change from winter to summer. For example, winter microbes use dead plant materials, whereas plant root exudates supply available C for summer microbes (Lipson et al., 2002; Schmidt et al., 2007). These changes in microbial communities changes might play key

roles in controlling annual patterns of nutrient cycling and plant N uptake in Arctic and alpine ecoysystems (Schmidt et al., 2007; Buckeridge and Grogan, 2008; Buckeridge et al., 2013).

In Arctic and alpine soils, microbial biomass and available N pools increase in winter, followed by the reduction in microbial biomass during winter–spring transition when the soil thaws (Brooks et al., 1998; Lipson et al., 1999; Schmidt and Lipson, 2004; Miller et al., 2009). ~~In alpine ecosystems, D~~decrease in microbial biomass is linked to a sudden rise in N availability during soils thawing, as observed in alpine ecosystems (Brooks et al., 1998; Lipson et al., 1999; Schmidt et al., 2007; Yang et al., 2016). The release of soluble N from microbial biomass during the soil thawing period provides an important available N source to plants, particularly in N-limited ecosystems (Lipson et al., 1999; Miller et al., 2009; Buckeridge and Grogan, 2010). However, despite ample evidence of soil microbial activity and nutrient mineralization during the winter and/or summer months in Arctic and alpine regions (Edwards et al., 2006; Schmidt et al., 2007; Miller et al., 2009; Edwards and Jefferies, 2013; Buckeridge et al., 2013), studies that explore the changes in microbial and N pools in alpine ecosystems during summer and winter across several years are few. Thus, the annual patterns of microbial biomass and N pools in alpine ecosystems and their responses to seasonal and interannual weather variations remain unclear.

In this study, we documented the seasonal dynamics of soil microbial biomass and available N for three years in an alpine meadow in the eastern part of Qinghai–Tibet Plateau of China to address the following questions: 1) What are soil microbial and available N dynamics during the growing and nongrowing seasons in the alpine meadow? 2) What are interannual patterns of soil microbial and available N dynamics in the alpine meadow? 3) What environmental factors

affect these dynamics? 4) What are the relationships between soil microbial biomass and available N pools in seasonally frozen ecosystems?

3 Material and methods

3.1 Site description

The study was performed in the alpine belt of Songpan County, which belongs to the Minshan Mountain in the eastern part of the Qinghai–Tibet Plateau, China. Accoding to the records from a meteorological station (33°1′ N, 103°41′ E, 3600 m a.s.l.) near the study area, the average monthly air temperatures range from −7.6 ℃ in January to 15.5 ℃ in August. The annual precipitation is 718 mm, ~~and~~ 70 % of which occurs from June to August. The region has no absolute frost-free period, and snowfall usually occurs from late September to early May. Persistent snow cover usually occurs from late December to early April, and the mean snow depth is 16.58 cm in the study area (Xu, unpublished data, collected in 2012, 2013). The alpine vegetation community has rich species composition, and dominated by different plant species during the growing season (i.e., during early May to late October according to the plant phenology observation in the alpine meadow from 2011 to 2013). Early flowering plants, such as *Primula sikkimensis*, *Androsace umbellate*, and *Caltha palustris*, dominate the community as soon as the snow melts; *Polygonum macrophyllum*, *Ranunculus tanguticus*, and *Carex melanocephala* dominate the middle growing season; and *Saussurea hieracioides* and *Gentiana sino-ornata* usually dominate the late growing season (Xu, unpublished data, collected from 2011 to 2013). The predominant soil type is mountain dark brown soil and Mat Cry-gelic Cambisols (i.e., silty loam inceptisol; Chinese Soil Taxonomy Research Group, 1995; Soil Survey Staff, 2014; Wang et al., 2016).

Study sites were located in an alpine meadow at Kaka Mountain (Fig. 1), which is a representative landscape in this region. Considering the soil spatial heterogeneity, three adjacent sites, approximately 100 m apart (centered at 32°59′ N, 103°40′ E, 3980 m a.s.l.) were selected. One site is located at the upper part of the alpine meadow, one at the middle part, and one at the lower part. Five replicates were collected from each site. The replicates from each site were 10 m apart from one another. The samples collected from the three sites (n = 15) at each sampling time were used for the statistical analyses. Given that plant roots are mainly distributed at 0–20 cm soil depth, soil sampling was only focused on this soil layer.

3.2 Soil sampling

Soil samples were collected on the 15th day of each month from April 2011 to October 2013. Overall, 31 sampling times were performed~~, and 15 soil samples were collected during each sampling time~~. The 1–2 cm layer of the surface material (i.e., O horizon, living plant roots and litter) of each soil sample was removed. During the cold periods (i.e., November to April), the samples were collected with a portable permafrost drill. The frozen soil samples were cut into little pieces (< 1 cm^3) with a knife and hammer, and large roots and sticks were removed. The soil samples collected during the warm seasons (i.e., May to October) were sieved to separate the plant materials and other fragments greater than 2 mm in diameter. The soils were then mixed and divided into three subsamples for further analysis. All the samples were processed at the laboratory of Chengdu Institute of Biology, CAS, within 2 days of sampling.

3.3 Soil temperature measurement

Soil temperatures were measured at the center of each sampled location. Soil temperatures at 10 cm depth were recorded

with DS1921G Thermochron iButton data loggers (DS1921G–F5, Maxim Integrated Products, Dallas Semiconductor Inc., Sunnyvale, CA, USA) at 1 h interval during the experimental period. Three iButton data loggers were placed at each site, and mean daily temperatures were then calculated from the data of the nine loggers. The mean temperature of the growing season was calculated by the mean daily temperatures from 1 May to 31 October, and that of the nongrowing season was calculated by the mean daily temperatures from 1 November to 30 April.

3.4 Soil water content and nutrient analyses

One subsample was used to measure gravimetric soil water content (SWC) after drying at 105 ℃ for 12 h. For the determination of total dissolved N (TDN) content, fresh soil subsamples (15 g) were measured into a beaker, and ~~placed into a sealed vacuum dryer along with another beaker containing 100 mL of chloroform. The samples were then subjected to vacuum treatment three times. A vacuum dryer was placed into the incubator under a temperature of 24 ℃ for 24 h and then subjected to vacuum treatment for approximately 30 min.~~ K_2SO_4 (0.5 M) was then added into the ~~chloroform-treated~~ soil samples with a soil weight-to-extractant volume (w/v) ratio of 1 : 5. The mixture was shaken for 1 h at 24 ℃. The extracted solution was filtered through filter paper (0.45 μm) and stored at −20 ℃ before determination (Lu, 2000; Jones and Willett, 2006). ~~Then, 10 mL of the extracted solution was placed into a test tube containing 10 mL of oxidant (NaOH-$K_2S_2O_8$ mixed solution). The resulting solution was subjected to water bath treatment at 120 ℃ for 90 min.~~ The TDN was then determined with an ultraviolet spectrophotometer. ~~For the determination of available inorganic N (NH_4^+–N and NO_3^-–N), the extracted treatment solution used was similar to that used for the TDN, except that it was not subjected to chloroform fumigation.~~ The NH_4^+–N and NO_3^-–N contents were determined via the indophenol blue

colorimetry (Sah, 1994) and ultraviolet spectrophotometry (Norman et al., 1985), respectively. Dissolved organic N (DON) was calculated by subtracting dissolved inorganic N (NH_4^+–N and NO_3^-–N) from TDN.

For the determination of the soil dissolved organic carbon (DOC), 10 g of fresh soil subsamples were shaken with 0.5 M K_2SO_4 at a 1: 5 w/v ratio for 1 h at 24 ℃, and the suspension was filtered at 0.45 μm under suction. The DOC values of the extracts were then measured through ultraviolet spectrophotometry (Lu, 2000; Jones and Willett, 2006).

3.5 Soil microbial biomass and community analyses

For the determination of soil microbial biomass C (MBC) and N (MBN), fresh soil subsamples (15 g) were measured into a beaker and placed into a sealed vacuum dryer along with another beaker containing 100 mL of chloroform. The samples were then subjected to vacuum treatment three times. A vacuum dryer was placed into the incubator under a temperature of 24 ℃ for 24 h and then subjected to vacuum treatment for approximately 30 min. K_2SO_4 (0.5 M) was added into the chloroform-treated soil samples with a soil weight-to-extractant volume (w/v) ratio of 1 : 5. The mixture was shaken for 1 h at 24 ℃. The extracted solution was filtered through filter paper (0.45 μm) and stored at −20 ℃ before determination (Lu, 2000; Jones and Willett, 2006). The extracted solution of non-chloroform-treated samples was made similar to that of chloroform-treated samples, except that it was not subjected to chloroform fumigation. The contents of C and N in the extracted solution were then measured through ultraviolet spectrophotometry (Lu, 2000; Jones and Willett, 2006). The MBC and MBN were then calculated by subtracting the C and N contents of non-chloroform-treated samples from that of chloroform-treated samples, respectively. ~~Soil microbial biomass C (MBC) and N (MBN) were determined via the chloroform-fumigation extraction method (Witt et al., 2000).~~ Correction factors of 0.45 for C

and 0.54 for N were used to convert the chloroform labile C and N to microbial C and N, respectively (Brookes et al., 1985; Wang et al., 2016).

The total colony-forming units (CFU) of bacteria, fungi, and actinomycetes were determined via the dilution-plate method (Li, 1996; Igbinosa, 2015). A total of 10 g of measured fresh soil subsamples were placed into a sterile jar, to which 90 mL of sterile distilled water was added. The jar was then covered with a sterile rubber plug and oscillated for 10 min for stock solution preparation. Serial diluent was made from the stock solution. The 10^{-5} and 10^{-6} dilution ratios of the serial diluent were selected for the determination of bacteria and actinomycetes, and 10^{-2} and 10^{-3} dilution ratios for fungi determination (Li, 1996). The selective mediums for bacteria, fungi, and actinomycetes were beef extract peptone agar, Sabouraud dextrose agar, and Gause synthetic agar medium, respectively (Li, 1996; Igbinosa, 2015). Soil diluent (1 mL) and medium (10 mL) at 45–50 ℃ were injected into the plates and cultured at 28 ℃ for 7–10 days for the bacteria and actinomycetes. Another medium with same components was prepared at 25 ℃ for 3–5 days for the fungi. The CFUs of different microbes were counted under a microscope (Li, 1996).

3.6 Statistical analyses

The normal distribution and homogeneity of variance of the sample datum were analyzed with SAS 9.2 software (SAS Institute Inc., 2008). The results met the basic requirements of analysis of variance~~variance analysis~~. Microbial and nutrient variables were analyzed to test the intra-annual differences between the growing season (i.e., data from May to October were used as a sample set; n = 90) and nongrowing season (i.e., data from November to April were used as a sample set; n = 90). Their interannual differences were also tested. Two-way ANOVA was performed via mixed-effects

model, with season and year specified as fixed effects. For the analysis of the microbial community shifts during the transition between nongrowing and growing seasons, differences in the number of bacteria, fungi, and actinomycetes between the late nongrowing season (i.e., in March) and early growing season (i.e., in May) were determined via two-way ANOVA. This procedure was performed for 2 years (2012 and 2013), and season and year specified were used as fixed effects. Pearson correlation analysis was then performed to analyze the correlation between MBC and SWC and that between MBC and DOC during the nongrowing and growing seasons. Significant results were determined at the $p < 0.05$ level, and Duncan's test was performed to analyze the significant results of the multiple comparisons to the interaction effects between season and year (SAS Institute Inc., 2008).

4 Results

4.1 Soil temperature and water content

In the alpine meadow, the mean soil temperatures (at 10 cm depth) were 6.01 ℃, 7.61 ℃, and 7.06 ℃ during the three growing seasons (May to October) from 2011 to 2013 and −1.76 °C and −2.17 °C during the two nongrowing seasons (November to April, Fig. 2). In addition, the soil was frozen (below 0 ℃) for 125 days on 2011–2012 and 165 days on 2012–2013. The soil was deeply frozen (below −5 °C) for 32 days on 2011–2012 and 36 days on 2012–2013. Significant seasonal and interannual differences in topsoil water contents (0–20 cm depth, SWC) were observed (Table 1). The SWC showed a decreasing trend during the growing season and increasing trend during nongrowing season (Fig. 3A), and SWC in the nongrowing season was significantly higher than that in the growing season (Fig. 3B). No significant difference was observed between the SWC mean values in the nongrowing season of 2011–2012 (64.73 % ± 2.22 %)

and those in the nongrowing season of 2012–2013 (65.68 % ± 4.03 %; $p > 0.05$; Fig. 3B). However, the SWC mean values in the growing seasons on 2011–2013 were significantly different ($p < 0.05$; Fig. 3B), and the lowest SWC was 46.43 % ± 2.28 % during 2012–2013.

4.2 Soil microbial biomass and community

Significant differences in MBC between seasons ($F = 860.28$, df = 1, $p = 0.00$) and years ($F = 4.46$, df = 2; $p = 0.01$) were observed in the soils of the alpine meadow (Table 1). The annual peak of MBC occurred in the late nongrowing season (March) then sharply decreased, indicating a diminishing trend during the growing season. The MBC reached a minimum value in the late growing season (September) then showed an increasing trend during the nongrowing season (Fig. 4A). However, a trend of significant decrease in MBC was observed in February when the soil temperatures were the lowest (below −5 °C). In addition, the MBC values in the nongrowing seasons were consistently higher than those in the growing seasons. The mean MBC value during the nongrowing season in 2012–2013 (i.e., 943.93 mg kg^{-1} ± 80.01 mg kg^{-1}) was significantly ($p < 0.05$) higher than those in the other seasons. Meanwhile, the mean MBC value during the growing season in 2012–2013 (i.e., 143.53 mg kg^{-1} ± 20.99 mg kg^{-1}) was the lowest (Fig. 4C). The MBC during the growing season had highly significant positive correlation with SWC ($p < 0.01$, $r = 0.62$; Table 2).

The soil MBN values had significant interannual differences ($F = 11.06$, df = 2; $p = 0.00$), but the seasonal differences among MBN values were nonsignificant ($F = 0.06$, df = 1; $p = 0.80$; Table 1). The seasonal and interannual dynamics of MBN were similar to those of MBC, and its annual peak generally occurs in April or May. Furthermore, no significant difference was observed between the mean MBN values in the growing season of 2013 and those in 2011–2012 ($p > 0.05$).

The lowest MBN value (72.06 mg kg^{-1} ± 5.93 mg kg^{-1}) was observed during the growing season in 2012–2013 (Fig. 4C).

Additionally, the microbial community comprised bacteria, fungi, and actinomycetes, showing a significant shift during the winter–spring transition (March to May; $p < 0.05$; Fig. 5). The number of bacteria in May was significantly higher ($p < 0.05$) than that in March, and the number of bacteria in May 2013 (i.e., 8.25×10^6 CFU g^{-1}) was significantly higher ($p < 0.05$) than that in 2012 (i.e., 7.22×10^6 CFU g^{-1}). The numbers of fungi and actinomycetes in March were significantly higher than that in May ($p < 0.05$). The number of fungi in March 2013 (4.33×10^4 CFU g^{-1}) was the highest, and no significant difference was observed between the number of actinomycetes in March 2012 and that in March 2013 ($p > 0.05$; Fig. 5).

4.3 Soil dissolved organic carbon

Significant interannual differences ($F = 5.50$, df = 2; $p = 0.01$) in soil DOC contents were observed, and the seasonal dynamics of DOC had no significant difference ~~from each other~~ ($F = 0.04$, df = 1; $p = 0.85$; Table 1). DOC peaks annually in May and shows a diminishing trend during the growing season and increasing trend during the nongrowing season (Fig. 6A). The DOC contents during the nongrowing season in 2011–2012 (174.27 mg kg^{-1} ± 32.59 mg kg^{-1}) and growing season in 2012–2013 (170.85 mg kg^{-1} ±41.19 mg kg^{-1}) had no significant differences ($p > 0.05$), but those were significantly lower than that in other seasons ($p < 0.05$; Fig. 6B). Furthermore, the DOC during the growing season had highly significant positive correlation with MBC ($p < 0.01$, $r = 0.64$; Table 2).

4.4 Soil available nitrogen

Soil ammonium N (NH_4^+–N) contents showed significant seasonal and interannual differences (F = 28.3, df = 1; p = 0.00 and F = 3.20, df = 2; p = 0.04; Table 1). The annual peak of the NH_4^+–N content occurred in the late nongrowing season (April), and then sharply reduced during the early growing season, and finally had an increasing trend during the nongrowing season (Fig. 7A). The NH_4^+–N content in the nongrowing season was significantly higher ($p < 0.05$) than that in the growing season. The NH_4^+–N content during the non-growing season in 2012–2013 (22.21 mg kg^{-1} ± 3.87 mg kg^{-1}) was significantly higher than that in 2011–2012 (17.23 mg kg^{-1} ± 3.85 mg kg^{-1}), and no significant difference was observed among the NH_4^+–N contents during the growing seasons in 2011–2013 ($p > 0.05$; Fig. 8).

Significant seasonal and interannual differences in soil nitrate N (NO_3^-–N) contents were observed (F = 4.34, df = 1; p = 0.04 and F = 3.28, df = 2; p = 0.04; Table 1). The NO_3^-–N content showed an increasing trend during nongrowing seasons and increased initially before decreasing during the growing seasons (Fig. 7B). Furthermore, a~~n obviously~~ decreasing trend of NO_3^-–N contents was observed during the soil thawing period (April to May). The NO_3^-–N contents peaked annually in June while that during the nongrowing season in 2011–2012 (7.64 mg kg^{-1} ± 1.12 mg kg^{-1}) was the lowest. No significant difference was observed among the NO_3^-–N contents of the other seasons ($p > 0.05$; Fig. 8).

The DON contents had significant interannual differences (F = 10.13, df = 2; p = 0.00), but their seasonal differences were not significant (F = 0.63, df = 1; p = 0.43; Table 1). In general, the peak DON content was observed in April or May, then sharply decreased during the middle and late growing season, and finally increased during the nongrowing season (Fig. 7C). Furthermore, the mean DON value during the growing season in 2012–2013 (7.53 mg kg^{-1} ± 1.74 mg kg^{-1}) was the lowest, and it was significantly lower than those in the other years ($p < 0.05$; Fig. 8).

5 Discussion

5.1 Seasonal microbial biomass and available nitrogen dynamics

The significant seasonal dynamics of the soil microbial biomass and available N pools were observed in the alpine meadow located in the eastern part of the Qinghai–Tibet Plateau for three years (Table 1; Figs. 4 and 7). Generally, the soil MBC and available N pools both increased at the beginning of the early nongrowing season, and this finding is consistent with the results of previous studies conducted in other Arctic and alpine ecosystems (Brooks et al., 1998; Lipson et al., 1999; Lipson et al., 2002; Edwards et al., 2006; Larsen et al., 2007, Buckeridge et al., 2010; Edwards and Jefferies, 2013). This period of active microbial activity and N mineralization might benefit~~ed~~ from substrates conducive for microbial growth, particularly those supplied by the fresh plant litter inputs in autumn (Lipson et al., 1999; Nemergut et al., 2005). However, a decline in soil MBC was observed during the deeply cold period (i.e., in February when soil temperatures were below – 5 ℃). This decline implied that the temperature threshold of the survival of these cold-adapted microbial communities was at least −5 °C. ~~Thus, an accumulation of inorganic and organic N pools occurred during the long and cold nongrowing seasons in these seasonally frozen ecosystems (Schimel and Mikan, 2005; Schmidt et al., 2007).~~

The annual peak of MBC generally occurred during the late nongrowing season while the mean soil temperatures were below 0 ℃. A modest reduction in MBC was observed in the onset of early soil thaw, and a steep decline in MBC occurred during the late soil-thawing period while the mean soil temperatures exceeded 0 ℃. This sharp decrease in MBC during the transition between nongrowing and growing seasons was similar to the changes of MBC in other Arctic

and alpine meadows during late winter and early spring (Lipson et al., 2002; Edwards et al., 2006). Previous studies suggested several factors that contribute to the decline of MBC during the soil thawing period. First, physical changes in soil during thawing can result in microbial cell death and release of solutes (Jefferies et al., 2010; Edwards and Jefferies, 2013). Second, depletion of soil available C and N can also lead to microbial reductions during soil thawing (Edwards et al., 2006; Buckeridge and Grogan, 2008). Furthermore, Edwards and Jefferies (2013) hypothesized that oxygen availability in soils may lead to MBC reductions because aerobic microbial growth can still be supported in winter. Anaerobic soil conditions are established as soils become flooded with liquid water during the late soil thaw. However, in our study, increases in DOC and inorganic N (NH_4^+–N and NO_3^-–N) contents was observed during the nongrowing season, implying that available C and N were relatively sufficient and might not restrict microbial activity during the winter–spring transition. This phenomenon may be closely related to the high plant community productivity in the eastern part of the Qinghai-Tibet Plateau. The aboveground biomass ranges from 299.8 g m^{-2} a^{-1} to 475.8 g m^{-2} a^{-1} in the alpine meadows on this region (Gao et.al, 2008; Yang et al., 2014) but 198 ± 73.8 g m^{-2} a^{-1} in the paramo grassland of Colombia (Hofstede et al., 1995) and ranges from 160 g m^{-2} a^{-1} to 230 g m^{-2} a^{-1} in the alpine meadows of the central Rocky mountains (Walker et al., 1994; Körner, 2003). Furthermore, the soil organic matter content in the alpine meadows of this region ranges from 69.7 g kg^{-1} to 112.4 g kg^{-1} (Wu and Onipchenko, 2005) but 12.8 g kg^{-1} in the Alaskan tundra (Körner, 2003) and ranges from 20.3 g kg^{-1} to 34.7 g kg^{-1} in the alpine meadows of the Alps and Colorado (Billings and Bliss, 1959; Körner, 2003).

Additionally, a significant difference was observed between the microbial community composition in the nongrowing

seasons and those in the growing seasons (Fig. 5). Winter microbial community was dominated by fungi, which is more adapted to cold temperatures and utilizes complex substrates (Lipson et al., 2002; Schadt et al., 2003). Apart from the fungi community, another important microbial community in winter soils is the actinomycetes. Furthermore, the number of bacteria significantly increased during the early growing season after the soils completely thawed. By contrast, number of fungi and that of actinomycetes declined considerably. This shift in the microbial community may lead to the sharp decline in MBC during soil thaw, possiblypartly because of the C investment per unit volume in fungal cells were threefold larger than that in bacteria cells (Buckeridge and Grogan, 2008).

In the present study, inorganic N and DON contents both showed an increasing trend during the nongrowing season, and this trend was closely related to high microbial biomassactivity in the soils of this region (Lipson et al., 1999; Matthew Robson et al., 2010). However, divergent dynamics among different forms of available N were observed during the growing season (Fig. 8). An obviously trend of increasing NH_4^+–N content was found during the early soil thaw. Furthermore, frequent and strong freeze–thaw cycles during this period may contribute to the release of unavailable NH_4^+–N from the organic and inorganic colloids in alpine soils (Freppaz et al., 2007). Snow melting during this period is may be an important source of NH_4^+–N (Williams and Tonnessen, 2000). At the start of the growing season, NH_4^+–N content sharply decreased, possibly partly because alpine meadow plants prefer NH_4^+–N (Jaeger et al., 1999; Gherardi et al., 2013). Moreover, strong microbial activity in the soil requires a large amount of NH_4^+–N at increasing temperature (Bowman, 1992; Schmidt and Lipson, 2004). As observed in other alpine regions (Brooks et al., 1997; Edwards et al., 2007), the NO_3^-–N had a sharp decline during the soil thaw in our study, mostly because a massive amount of NO_3^-–N

might have run off with the snow melt water. The NO_3^-–N content first increased during the early growing season and then decreased during the middle growing season as the NH_4^+–N content decreased. Meanwhile, DON content slightly decreased during the early and middle growing season and sharply decreased during the late growing season as both NH_4^+–N and NO_3^-–N were exhausted. These results implied that although the DON may not be the main source of N pools for plants, it is an effective supplement of the available N pool. Furthermore, the seasonal dynamics of different available N pools showed significant complementarity with the nutrient supply process and will play a crucial role in maintaining abundant biodiversity of alpine meadow ecosystem (Kahmen et al., 2006; Ashton et al., 2010~~Qin et al., 2003; Petchey and Gaston, 2006~~).

5.2 Interannual microbial biomass and available nitrogen dynamics

Significant interannual differences in microbial biomass and available N were observed across the study years. For example, the MBC and NH_4^+–N contents during the nongrowing season in 2012–2013 were significantly higher than those in 2011–2012, and MBC during the growing season in 2012–2013 was the lowest among the growing seasons (Figs. 4 and 8). Furthermore, significant positive correlation between MBC and SWC was observed during the growing season (Table 2). This result suggested that interannual variability of soil water conditions is an important environmental driver that affects the microbial biomass in alpine meadows. First, low soil moisture in the growing season causes a decline in plant productivity (Körner, 2003), resulting in a decline of C substrates supplied by plant root exudates and litters. Second, low soil moisture in summer leads to an increased oxidation in the surface soil, thus exerting significant influence on the microbial communities (Blodau et al., 2004), and some of these influences are retained during winter

(Edwards and Jefferies, 2013). Notably, the nongrowing season in 2011–2012 was warmer and drier than that in 2012–2013, which might accompanied with more frequent freeze–thaw cycles during the early period of this season (Mellander et al., 2007; Henry, 2008). These environmental variations might contribute to the reduction in soil microbial biomass during the nongrowing season (Larsen et al., 2002; Yanai et al., 2004; Mellander et al., 2007; Henry, 2008). Although the extent of the influence of these environmental factors on soil microbial biomass cannot be verified, our ~~monitoring~~ results suggested that soil moisture and temperature are two important environmental factors influencing the interannual dynamic of soil microbial biomass.

In the alpine meadow, organic matter decomposition and nutrient mineralization caused by soil microbial activity during a long cold season play a crucial role in accumulating soil inorganic N pool (Hidy, 2003; Rinnan et al., 2007), and the microorganism itself is also an important soil organic N pool (Lipson et al., 2002). Thus, the interannual pattern of the soil microbial biomass largely affects the interannual change of soil N pool. Soil NH_4^+–N and DON had a consistent interannual variation with soil MBC during the nongrowing season. However, they showed a divergent interannual pattern during the growing season, possibly~~partly~~ because of the plant and microbe uptakes and leaching effects. Meanwhile, for the NO_3^-–N, relatively small interannual variability was observed. In addition, the interannual variability of precipitation affected the interannual pattern of available inorganic N pool in the soil. The snow melt is not only an important supplement for the NH_4^+–N pool (Williams and Tonnessen, 2000) but also a cause of a mass of NO_3^-–N losses during the soil-thawing period (Brooks et al., 1997; Edwards et al., 2007). Therefore, such interannual variations in the microbial and nutrient dynamics may become more common and pronounced in the alpine meadow in the eastern part

of the Qinghai–Tibet Plateau as a result of multiple impacts of climate change, particularly increasing extreme weather events, such as winter warming and heterogeneous precipitation (Edwards and Jefferies, 2013).

6 Conclusions

A trend of increasing soil MBC and available N pools was found in nongrowing seasons. A sharp decline in MBC was also observed during the soil–thawing period. Microbial activity may not be restricted by the soil available C and N in the time of soil thaw. However, a shift in microbial community induced by changing temperatures may largely contribute to this decline in MBC. Different forms of available N pools showed a divergent decreasing pattern during the growing season, suggesting that a significantly complementary pattern of nutrient supply exists among different N pools. Furthermore, the soil microorganism not only has a close correlation with the accumulation of inorganic N pools but also is an important soil organic N pool itself. Thus, the interannual dynamics of soil microbial biomass substantially affects the interannual differences among soil available N pools. According to our ~~monitoring~~ results, soil temperature and water condition are the primary environmental factors driving the seasonal and interannual dynamics of soil microbial biomass and available N pools. Owing to the changing climates of alpine ecosystems, soil microbial activities and nutrient supply patterns are expected to change further. These changes play an important role in the productivity and biodiversity of these regions. Long-term integrative studies on intra- and interannual variations of microbial and nutrient dynamics have important implications for ecosystem functions and their responses to environmental changes. Combined with some objective experimental studies, these research results can provide crucial insights into the biogeochemical cycles and functions of ecosystems in the eastern part of the Qinghai–Tibet Plateau, and their potential responses to the future

climate change.

7 Data availability

The data set related to this study has been provided as a supplement.

8 Author contribution

Fusun Shi, Ning Wu, and Yan Wu designed the experiments; Bo Xu and Jinniu Wang carried field experiments out; Bo Xu prepared the manuscript with contributions from all co-authors.

9 Competing interests

The authors declare that they have no conflict of interest.

10 Acknowledgements

The study was funded by the Key Research and Development Plan of China (2016YFC0501805).

11 References

Ashton, I. W., Miller, A. E., Bowman, W. D., and Suding, K. N.: Niche complementarity due to plasticity in resource use: plant partitioning of chemical N forms. Ecology, 99: 3252–3260, 2010.

Billings, W.D., and Bliss, L.C.: An alpine snowbank environment and its effects on vegetaiton, plant development, and productivity. Ecology, 40, 388–397, 1959.

Blodau, C., Basiliko, N., and Moore, T. R.: Carbon turnover in peatland mesocosms exposed to different water table levels. Biogeochemistry, 67, 331–351, 2004.

Bowman, W. D.: Inputs and storage of nitrogen in winter snowpack in an alpine ecosystem. Arct. Alp. Res., 24, 211–

215, 1992.

Brookes, P. C., Landman, A., Pruden, G., and Jenkinson, D. S.: Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil, Soil Biol. Biochem., 17, 837–842, 1985.

Brooks, P. D., Schmidt, S. K., and Williams M.W.: Winter production of CO_2 and N_2O from Alpine tundra: Environmental controls and relationship to inter–system C and N fluxes. Oecologia, 110, 403–413, 1997.

Brooks, P. D., Williams, M. W., and Schmidt, S. K.: Microbial activity under alpine snowpacks, Niwot Ridge, Colorado. Biogeochemistry, 32, 93–113, 1996.

Brooks, P. D., Williams, M.W., and Schmidt, S. K.: Inorganic nitrogen and microbial biomass dynamics before and during spring snowmelt. Biogeochemistry, 43, 1–15, 1998.

Buckeridge, K. M., and Grogan, P.: Deepened snow alters soil microbial nutrient limitations in arctic birch hummock tundra. Appl. Soil Ecol., 39, 210–222, 2008.

Buckeridge, K. M., and Grogan, P.: Deepened snow increases late thaw biogeochemical pulses in mesic low arctic tundra. Biogeochemistry, 101, 105–121, 2010.

Buckeridge, K. M., Banerjee, S., Siciliano, S. D., and Grogan, P.: The seasonal pattern of soil microbial community structure in mesic low arctic tundra. Soil Biol. Biochem., 65, 338–347, 2013.

Chinese Soil Taxonomy Research Group: Chinese Soil Taxonomy, China Agriculture Scientech Press, 1995.

Deming, J. W.: Psychrophiles and polar regions. Curr. Opin. Microbiol. 5, 301–309, 2002.

Devi, N. B., and Yadava, P. S.: Seasonal dynamics in soil microbial biomass C, N and P in a mixed–oak forest ecosystem

of Manipur, North–east India. Appl. Soil Ecol., 31, 220–227, 2006.

Edwards, A. C., Scalenghe, R., and Freppaz, M.: Changes in the seasonal snow cover of alpine regions and its effect on soil processes: A review. Quatern. Int., 162, 172–181, 2007.

Edwards, K.A., and Jefferies, R.L.: Inter–annual and seasonal dynamics of soil microbial biomass and nutrients in wet and dry low–Arctic sedge meadows. Soil Biol. Biochem., 57, 83–90, 2013.

Edwards, K.A., McCulloch, J., Kershaw, G.P., and Jefferies, R.L.: Soil microbial and nutrient dynamics in a wet Arctic sedge meadow in late winter and early spring. Soil Biol. Biochem., 38, 2843–2851, 2006.

Freppaz, M.,Williams, B.L., Edwards, A.C., Scalenghe, R., and Zanini, E.: Labile nitrogen, carbon, and phosphorus pools and nitrogen mineralization and immobilization rates at low temperatures in seasonally snow–covered soils. Biol. Fert. Soils, 43, 519–529, 2007.

Gao, Y. H., Chen, H., Luo, P., Wu, N. Wang, G. X.,: Effect of grazingin tensity on biomass of alpine meadow and its allocation in the northwestern sichuan. J. Ecol. Rural Environ., 24, 26–32, 2008.

Gherardi, L. A., Sala, O. E., and Yahdjian, L.: Preference for different inorganic nitrogen forms among plant functional types and species of the Patagonian steppe. Oecologia, 173, 1075–1081, 2013.

Henry H.A.L.: Climate change and soil freezing dynamics: historical trends and projected changes. Climatic Change, 87, 421–434, 2008.

Hidy G.M.: Snowpack and precipitation chemistry at high altitudes. Atmos. Environ., 37, 1231–1242, 2003.

Hofstede, R. G. M., Chilito, E. J., Sandovals, E. M.,: Vegetative structure, microclimate, and leaf growth of a páramo

tussock grass species, in undisturbed, burned and grazed conditions. Vegetatio, 119, 53–65, 1995.

Igbinosa, E.O.: Effect of cassava mill effluent on biological activity of soil microbial community. Environ. Monit. Assess., 187, 4651–4651, 2015.

Jaeger C.H., Monson R.K., Fisk M.C., and Schmidt S.K.: Seasonal partitioning of nitrogen by plants and soil microorganisms in an alpine ecosystem. Ecology, 80, 1883–1891, 1999.

Jefferies, R.L., Walker, N.A., Edwards, K.A., and Dainty, J.: Is the decline of soil microbial biomass in late winter coupled to changes in the physical state of cold soils? Soil Biol. Biochem., 42, 129–135, 2010.

Jones, D.L., and Willett, V.B.: Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biol. Biochem., 38, 991–999, 2006.

Kahmen, A., Renker, C., Unsicker, S. B., and Buchmann, N.: Niche complementarity for nitrogen: an explanation for the biodiversity and ecosystem functioning relationship? Ecology, 87: 1244–1255, 2006.

Körner, C.: Alpine Plant Life: Functional Plant Ecology of High Mountain Ecosystems. Springer, 2003.

Larsen, K. S., Grogan, P., Jonasson, S., and Michelsen, A.: Respiration and Microbial Dynamics in Two Subarctic Ecosystems during Winter and Spring Thaw: Effects of Increased Snow Depth. Arct. Antarct. Alp. Res., 39, 268–276, 2007.

Larsen, K.S., Jonasson, S., and Michelsen, A.: Repeated freeze–thaw cycles and their effects on biological processes in two arctic ecosystem types. Appl. Soil Ecol., 21, 187–195, 2002.

Li, F. D.: Experimental technique in agricultural microbiology. China Agriculture Press, Beijing, 1996.

Lipson, D. A., Schadt, C.W., and Schmidt, S. K.: Changes in soil microbial community structure and function in an

alpine dry meadow following spring snow melt. Microb. Ecol., 43, 307–314, 2002.

Lipson, D. A., Schmidt, S. K., and Monson, R. K.: Links between microbial population dynamics and nitrogen availability in an alpine ecosystem. Ecology, 80, 1623–1631, 1999.

Lu R. K.: Soil and Agro–Chemical Analytical Methods. Beijing: China Agricultural Science and Technology Press, 2000.

Matthew Robson, T., Baptist, F., Clement, J. C., and Lavorel, S.: Land use in subalpine grasslands affects nitrogen cycling via changes in plant community and soil microbial uptake dynamics. J. Ecol., 98, 62–73, 2010.

Mellander, P. E., Lofvenius, M. O., and Laudon H.: Climate change impact on snow and soil temperature in boreal Scots pine stands. Climatic Change, 85, 179–193, 2007.

Mikan, C. J., Schimel, J. P., and Doyle, A. P.: Temperature controls of microbial respiration in arctic tundra soils above and below freezing. Soil Biol. Biochem., 34, 1785–1795, 2002.

Miller, A. E., Schimel, J. P., Sickman, J. O., Skeen, K., Meixner, T., and Melack, J. M.: Seasonal variation in nitrogen uptake and turnover in two high–elevation soils: mineralization responses are site–dependent. Biogeochemistry, 93, 253–270, 2009.

Murata, T., Tanaka, H., Yasue, S., Hamada, R., Sakagami, K., and Kurokawa, Y.: Seasonal variations in soil microbial biomass content and soil neutral sugar composition in grassland in the Japanese Temperate Zone. Appl. Soil Ecol., 11, 253–259, 1999.

Nemergut, D. R., Costello, E. K., Meyer, A. F., Pescador, M.Y., Weintraub, M. N., and Schmidt, S. K.: Structure and function of alpine and arctic soil microbial communities. Res. Microbiol., 156, 775–784, 2005.

Norman, R. J., Edberg, J. C., and Stucki, J. W.: Determination of Nitrate in Soil Extracts by Dual–wavelength Ultraviolet Spectrophotometry, Soil Sci. Soc. Am. J., 49, 1182–1185, 1985.

Oquist, M. G., Sparrman, T., Klemedtsson, L., Drotz, S. H., Grip, H., Schleucher, J., and Nilsson, M.: Water availability controls microbial temperature responses in frozen soil CO_2 production. Global Change Biol., 15, 2715–2722, 2009.

Panikov, N. S., Flanagan, P. W., Oechel, W. C., Mastepanov, M. A., and Christensen, T. R.: Microbial activity in soils frozen to below −39 °C. Soil Biol. Biochem., 38, 785–794, 2006.

~~Petchey, O. L., and Gaston, K. J.: Functional diversity: back to basics and looking forward. Ecol. Lett., 9, 741–758, 2006.~~

~~Qin, G. L., Du, G. Z., Luo, Y. J., Dong, G. S., and Ma, J. J.: A reexamination of the relationships among phenological complementarity, species diversity, and ecosystem function. Bot. Bull. Acad. Sin., 44, 239–244, 2003.~~

Rinnan, R., Michelsen, A., Baath, E., and Jonasson S.: Mineralization and carbon turnover in subarctic heath soil as affected by warming and additional litter. Soil Biol. Biochem., 39, 3014–3023, 2007.

Robertl, J., Nalan, W., Katea, E., and Jack, D.: Is the decline of soil microbial biomass in late winter coupled to changes in the physical state of cold soils? Soil Biol. Biochem., 42, 129–135, 2010.

Sah, R. N.: Nitrate–Nitrogen Determination–A Critical Review, Commun. Soil Sci. Plan., 25, 2841–2869, 1994.

SAS Institute Inc.: SAS 9.2 user's guide, Carolina, USA, 2008.

Schadt, C.W., Martin, A. P., Lipson, D. A., and Schmidt, S.K.: Seasonal dynamics of previously unknown fungal lineages in tundra soils. Science, 301, 1359–1361, 2003.

~~Schimel, J. P., and Mikan, C.: Changing microbial substrate use in Arctic tundra soils through a freeze–thaw cycle. Soil Biol. Biochem., 37, 1411–1418, 2005.~~

带格式的: 缩进: 左侧: 0 厘米, 首行缩进: 0 字符

Schmidt, S. K., and Lipson, D. A.: Microbial growth under the snow: Implications for nutrient and allelochemical availability in temperate soils. Plant Soil, 259, 1–7, 2004.

Schmidt, S. K., Costello, E. K., Nemergut, D. R., Cleveland, C. C., Reed, S. C., Weintraub, M. N., Meyer, A. F., and Martin, A. M.: Biogeochemical consequences of rapid microbial turnover and seasonal succession in soil. Ecology, 88, 1379–1385, 2007.

Soil Survey Staff: Keys to Soil Taxonomy, 12th ed. USDA-Natural Resources Conservation Service, Washington, DC, 2014.

Walker, M. D., Webber, P. I., Arnold, E. H., Ebert-May, D.,: Effects of interannual climate variation on aboveground phytomass in alpine vegetation. Ecology, 75, 393–408, 1994.

Wang J., Xu B., Wu Y., Gao J., and Shi F.: Flower litters of alpine plants affect soil nitrogen and phosphorus rapidly in the eastern Tibetan Plateau. Biogeosciences, 13, 5619–5631, 2016.

Williams, M.W., and Tonnessen, K. A.,: Critical loads for inorganic nitrogen deposition in the Colorado Front Range, USA. Ecol. Appl., 10, 1648–1665, 2000.

~~Witt, C., Gaunt, J.L., Galicia, C. C., Ottow, J. C. G., and Neue, H. U.: A rapid chloroform–fumigation extraction method for measuring soil microbial biomass carbon and nitrogen in flooded rice soils. Biol. Fert. Soils, 30, 510–519, 2000.~~

Wu, Y., and Onipchenko, V. G.: The structure of plant communities according to soil properties in the eastern Tibetan plateau. Trans. Tebe. State Bios. Rese. Russ., 30, 57–73, 2005.

Yanai, Y., Toyota, K., and Okazaki M.: Effects of successive soil freeze–thaw cycles on soil microbial biomass and

organic matter decomposition potential of soils. Soil Sci. Plant Nutr., 50: 821–829, 2004.

Yang, X. X., Ren, F., Zhou, H. K., He, J. S.,: Responses of plant community biomass to nitrogen and phosphorus additions in an alpine meadow on the Qinghai–Xizang Plateau. Chin. J. Plant Ecol., 38, 159–166, 2014.

Yang, Z., Gao, J., Yang, M., and Sun, Z.: Effects of freezing intensity on soil solution nitrogen and microbial biomass nitrogen in an alpine grassland ecosystem on the Tibetan Plateau, China. J Arid Land, 8, 749–759, 2016.

Table

Table 1. Results from two-way ANOVA comparing growing season (May to October) and nongrowing season (November to April) values across three years of study for SWC, MBC, MBN, DOC, $NH4^+$–N, NO_3^-–N, and DON in the alpine meadow.

Variable	Source	df	*F*	*p*
SWC	Year	2	6.79	0.00
	Season	1	180.62	0.00
	Year × season	2	18.29	0.00
MBC	Year	2	4.46	0.01
	Season	1	860.28	0.00
	Year × season	2	61.67	0.00
MBN	Year	2	11.06	0.00
	Season	1	0.06	0.80
	Year × season	2	20.79	0.00
DOC	Year	2	5.50	0.01
	Season	1	0.04	0.85
	Year × season	2	14.73	0.00
$NH4^+$–N	Year	2	3.20	0.04
	Season	1	28.3	0.00
	Year × season	2	0.39	0.53
NO_3^-–N	Year	2	3.28	0.04
	Season	1	4.34	0.04
	Year × season	2	0.18	0.67
DON	Year	2	10.13	0.00
	Season	1	0.63	0.43
	Year × season	2	6.40	0.01

Table 2. Pearson correlations of MBC between SWC and DOC during growing and nongrowing seasons

MBC	SWC	DOC
Growing season	0.62 **	0.64 **
Nongrowing season	0.35 **	0.12 ns

Note: ns, no significant difference; **, $p < 0.01$.

Figure legends

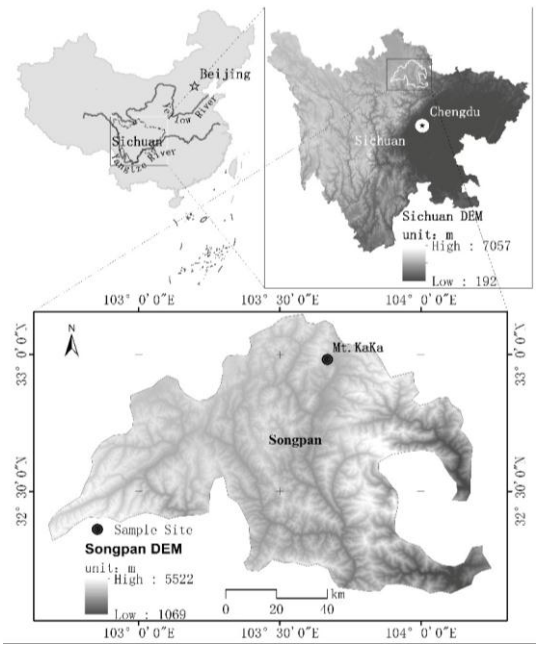

Fig. 1. Location of the study site

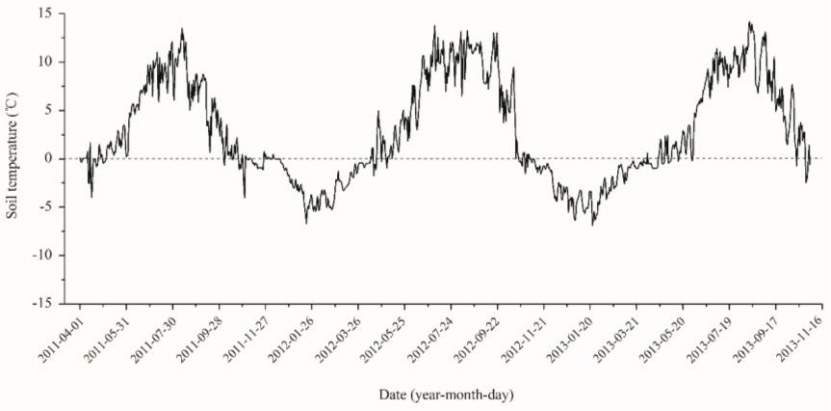

Fig. 2. Mean daily soil temperature in the alpine meadow from April 2011 to October 2013. Thermochron iButton data loggers were placed at 10 cm soil depth to obtain automatic readings every 1 h, and the mean daily soil temperature was calculated every day.

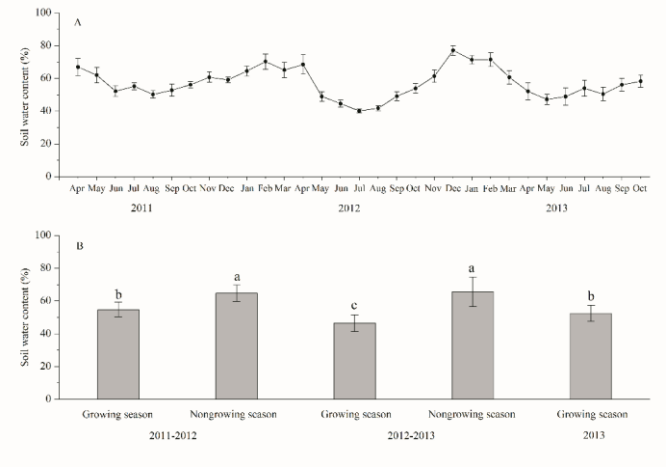

Fig. 3. Dynamics of soil water content (A; mean ± s.e.; n = 15) and its seasonal and interannual differences (B; mean ± s.e.; n = 90) from 2011 to 2013.

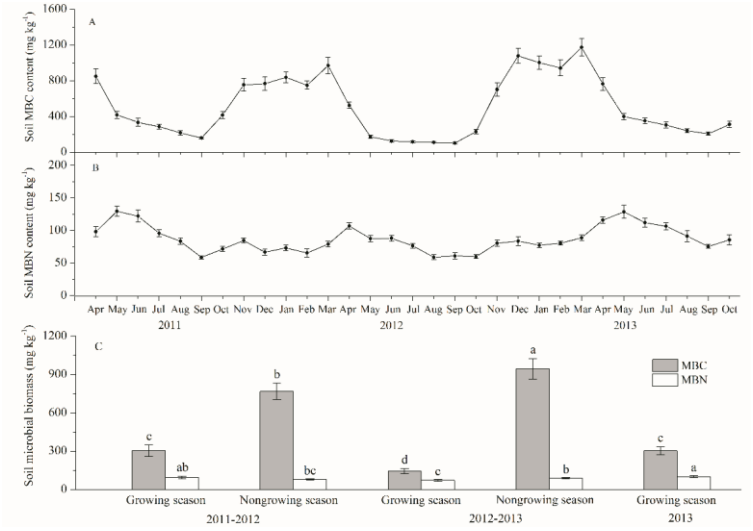

Fig. 4. Dynamics of microbial biomass C and N (A and B; mean ± s.e.; n = 15), and their seasonal and interannual differences (C; mean ± s.e.; n = 90) from April 2011 to October 2013 (mean ± s.e.; n = 90). The sampling time was on the 15th day of each month during the growing season from May to October, and during the nongrowing season from November to April next year. Seasons and years were compared using two-way ANOVA, and different lowercase letters indicate significant differences of the interaction effects between season and year determined via Duncan test ($p < 0.05$).

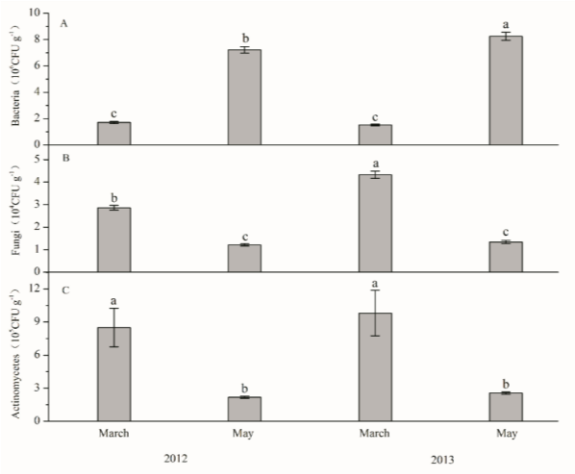

Fig. 5. Changes in the number of bacteria (A), fungi (B), and actinomycetes (C) during the transition between freezing and thawing periods (mean ± s.e.; $n = 15$). The sampling time during the freezing period was on 15 March and during the thawing period was on 15 May each year. Different lowercase letters indicate significant differences of the interaction effects between season and year according to two-way ANOVA ($p < 0.05$).

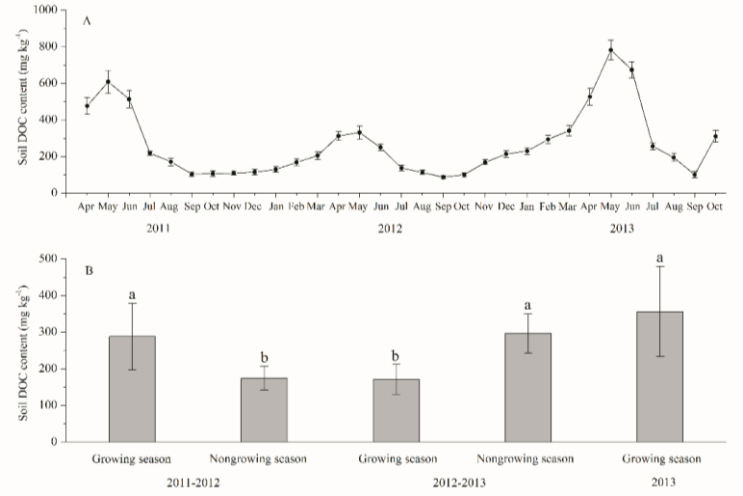

Fig. 6. Dynamics of dissolved organic C (A; mean ± s.e.; n = 15) and its seasonal and interannual differences (B; mean ± s.e.; n = 90) from 2011 to 2013.

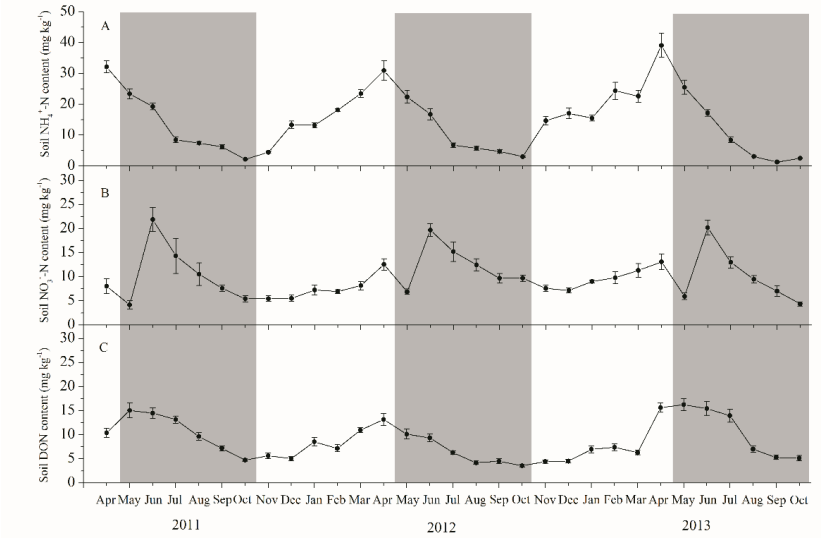

Fig. 7. Dynamics of NH_4^+–N(A), NO_3^-–N(B), and DON(C) in soils of the alpine meadow from April 2011 to October 2013 (mean ± s.e.; n = 15).

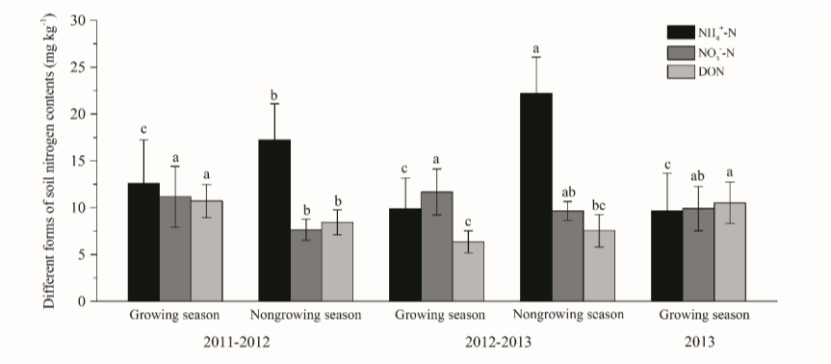

Fig. 8. Changes in NH_4^+–N, NO_3^-–N, and DON of growing and nongrowing seasons from 2011 to 2013 (mean ± s.e.; n =90). The sampling time was on the 15th day of each month from May to October during the growing season and during the nongrowing season from November to April next year. Seasonal and interannual differences were compared using two-way ANOVA. Different lowercase letters indicate significant differences of the interaction effects between season and year determined via Duncan test ($p < 0.05$).